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Utilization of Dissolved Nitrogen by Heterotrophic Bacterioplankton: Effect of Substrate C/N Ratio

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The significance of dissolved combined amino acids (DCAA), dissolved free amino acids (DFAA), and dissolved DNA (D-DNA) as sources of C and N for marine bacteria in batch cultures with variable substrate C/N ratios was studied. Glucose, ammonium, alanine, and phosphate were added to the cultures to produce C/N ratios of 5, 10, and 15 and to ensure that phosphorus was not limiting. Maximum bacterial particulate organic carbon production (after 25 h of incubation) was inversely correlated with the C/N ratio: with the addition of identical amounts of carbon, the levels of production were 9.0-, 10.0-, and 11.1-fold higher at C/N ratios of 15, 10, and 5, respectively, relative to an unamended control. The bacterial growth efficiency increased from 22% (control cultures) to 44 to 53% in the cultures with manipulated C/N ratios (C/N-manipulated cultures). Net carbon incorporation from DCAA, DFAA, and D-DNA supported on average 19, 4, and 3% (control cultures and cultures to which only phosphate was added [+P cultures]) and 5, 4, and 0.3% of the particulate organic carbon production (C/N-manipulated cultures), respectively. In the C/N-manipulated cultures, a 2.6- to 3.4-fold-higher level of incorporation of DCAA, relative to that in the control cultures, occurred. Incorporation of D-DNA increased with the substrate C/N ratio, suggesting that D-DNA mainly was a source of N to the bacteria. Organic N (DCAA, DFAA, and D-DNA) sustained 14 to 49% of the net bacterial N production. NH_4^+ was the dominant N source and constituted 55 to 99% of the total N uptake. NO_3^- contributed up to 23% to the total N uptake but was released in two cultures. The studied N compounds sustained nearly all of the bacterial N demand. Our results show that the C/N ratio of dissolved organic matter available to bacteria has a significant influence on the incorporation of individual compounds like DCAA and D-DNA.

Aquatic bacteria are capable of taking up a variety of dissolved organic nitrogen (DON) compounds that may be sources of both nitrogen and carbon for biosynthesis or may be used as sources of energy. However, results regarding the importance of individual DON species to total bacterial nitrogen demand are inconsistent. Uptake of dissolved free amino acids (DFAA) has been estimated to meet up to 37% (23) or >100% (4) of the bacterial nitrogen requirement in natural oceanic and estuarine waters. These observations have been confirmed with bacterial batch cultures, in which DFAA sustained up to 76% (7) or >100% (11, 13) of the bacterial nitrogen demand. Another source of amino nitrogen for bacteria is dissolved combined amino acids (DCAA). Uptake of DCAA has been found to account for a small percentage to >100% of the total bacterial nitrogen incorporation (1, 12, 18, 22, 26). Reported values concerning the significance of DCAA to aquatic bacteria are difficult to compare, however, since different procedures for measurements of concentrations and uptake rates have been applied. Among other DON compounds used by marine bacteria, dissolved DNA (D-DNA) has been estimated to supply 5%, or less, of the bacterial nitrogen demand (11, 18).

Simultaneously with uptake of DON, bacteria may utilize dissolved inorganic nitrogen (DIN), but it is difficult to predict whether DON or DIN will be the major nitrogen source. NH_4^+ and DFAA were found to be equally important as bacterial nitrogen sources in the subarctic Pacific when typical DFAA concentrations occurred (13). In another study of the same locality, NH_4^+ was utilized only when the DFAA concentrations were very low (15). Goldman and Dennett (7) found that whether NH_4^+ or DFAA is the dominant nitrogen source in marine batch cultures depends on the bacterial growth rate and the C/N ratio of the substrate. In most comparisons of DON and DIN assimilation, DFAA are assumed to be the dominant natural source of organic nitrogen. As mentioned above, however, recent studies suggest that DCAA can be of similar importance to pelagic bacteria (11, 18, 22).

In a concurrent paper, bacterial utilization of naturally occurring DCAA, DFAA, D-DNA, and DIN at three different localities has been described (18). In order to examine how utilization of these nitrogen species was influenced by changes in the substrate C/N ratio, nitrogen and carbon fluxes of DCAA, DFAA, D-DNA, and DIN at different C/N ratios in bacterial batch cultures from one of the localities, Santa Rosa Sound, were determined. The results indicate that utilization of primarily DCAA can be stimulated by changes of the natural C/N ratio of seawater.

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MATERIALS AND METHODS

Experimental design. Seawater for bacterial batch cultures was collected in Santa Rosa Sound, a shallow, coastal locality

TABLE 1. Experimental setup and substrate additions

Batch no. (culture type)[a]	Phosphate concn (μM)	Glucose concn (μM)	Ammonium concn (μM)	Alanine concn (μM)	C/N/P ratio (resulting from addition)
1 + 2 (control)	0	0	0	0	
3 + 4 (+P)	5	0	0	0	
5 + 6 (C/N = 5)	5	15	16.7	3.3	5:1:0.25
7 + 8 (C/N = 10)	5	15	6.7	3.3	10:1:0.25
9 + 10 (C/N = 15)	5	15	3.3	3.3	15:1:0.25

[a] All batch cultures were prepared from 10% (1.0-μm pore size) and 90% (0.2-μm pore size) filtered Santa Rosa Sound water.

in northwestern Florida, in April 1991. The salinity and water temperature at the sampling time were 20 ppt and 25°C. The seawater was filtered through 0.22- and 1.0-μm-pore-size filter cartridges (Millipore), and the differentially filtered samples were mixed in a 9:1 ratio, respectively. After filtration and mixing, the batch cultures were transferred to duplicate 10-liter carboys and incubated at 25°C in the dark. Subsamples were taken at 8- to 9-h intervals for measurements of various bacterial growth parameters. The carboys were shaken before each subsampling.

Substrate carbon-to-nitrogen ratios of the cultures were manipulated by addition of ammonium (NH_4Cl), glucose, and alanine (Table 1). Addition of the substrates led to final C/N ratios of 5, 10, and 15. Enrichment with glucose and alanine equaled 90 and 10 μmol of C per liter, or 1,080 and 120 μg of C per liter, respectively. To ensure that the bacterial growth was not limited by phosphorus, 5 μM phosphate (KH_2PO_4) was added to all cultures except the control.

Microbiological and chemical analyses. At each sampling, subsamples for different analyses were taken. Details of the analytical procedures are described by Kroer et al. (18). The following parameters were measured.

(i) Concentrations of DCAA. After a liquid-phase acid hydrolysis, individual DCAA were assayed as *o*-phthaldialdehyde derivatives by high-pressure liquid chromatography (HPLC). Concentrations of DFAA were subtracted from the measured DCAA concentrations. Uptake or release of DCAA was determined from changes of the ambient concentrations.

(ii) Assimilation (respiration and incorporation) of DFAA. Carbon and nitrogen flux of DFAA was estimated from the uptake of four ^{14}C-labelled amino acids (glutamic acid, serine, glycine, and alanine) and ambient DFAA pools, measured by HPLC. Addition of the ^{14}C-labelled amino acids increased the natural DFAA concentrations by 1.1 nM. Turnover times of the four amino acids were assumed to be representative of those of the ambient DFAA pools.

(iii) Net assimilation of D-DNA. *Escherichia coli* DNA was labelled by nick translation with [^{3}H]dATP and added to water samples at a concentration of 1.6 μg/liter. After incubation periods of 3 to 4 h, the bacteria were filtered onto 0.22-μm-pore-size membrane filters and radioassayed. Concentrations of natural D-DNA were measured after a fluorescence labelling according to the method of Karl and Bailiff (12). The turnover times determined from [^{3}H]DNA were assumed to represent bacterial turnover of natural D-DNA.

(iv) Bacterial production (from incorporation of [^{3}H]L-leucine) and abundance (acridine orange direct counts). Factors for conversion of leucine incorporation into bacterial carbon production are given in a footnote to Table 2.

(v) Particulate organic carbon (POC) and particulate organic nitrogen (PON). Volumes (200 to 400 ml) of water were filtered through Whatman GF/F filters, which were assayed on a Carlo Erba NA 1500 CHN Analyzer.

(vi) Dissolved organic carbon (DOC). Concentrations of DOC were measured on a Shimadzu TOC 5000 Analyzer with a Pt catalyst.

Ammonium, nitrite, and nitrate concentrations were measured by using standard autoanalyzer procedures.

Carbon and nitrogen budgets. Bacterial carbon and nitrogen budgets were calculated for the initial 25 h of incubation, when there were actively growing bacteria in all cultures. Incorporation of carbon and nitrogen from DCAA was determined from changes in concentrations by using linear regressions between 0 and 25 h. Incorporation of DFAA and D-DNA was determined from the isotope-based net assimilation rates. The carbon and nitrogen contents of DCAA and DFAA were based on the compositions of the individual amino acids. For D-DNA, carbon and nitrogen contents of 33 and 16%, respectively, were assumed.

RESULTS

Abundance and production of bacteria. The bacterial populations reached maximum densities after 25 h, except in the cultures to which only phosphate was added (+P cultures), in which the numbers were still increasing at 33 h (Fig. 1A). The bacterial populations increased from 0.4×10^9 cells per liter at the start to a maximum abundance of 0.9×10^9 to 5.5×10^9 cells per liter (values for the control cultures and the cultures for which the C/N ratio was 5 [C/N = 5 cultures], respectively). Incorporation of leucine was at low levels during the initial 8 h, after which it increased considerably (Fig. 1B). In the cultures with manipulated C/N ratios (C/N-manipulated cultures), the incorporation peaked at 25 h, but in the control and +P cultures, it was still increasing at 33 h. Relative to the control cultures, 9.5-, 10.8-, and 11.1-fold-higher levels of incorporation were observed in the C/N = 15, C/N = 10, and C/N = 5 cultures at 25 h, respectively. In the +P cultures, a 2.7-fold-higher level of incorporation occurred.

POC, PON, and DOC. The production of bacterial carbon and nitrogen increased significantly after 17 h in all cultures (Fig. 1C and D). The maximum POC content was 11.1-, 10.1-, and 9.0-fold larger in the C/N = 5, C/N = 10, and C/N = 15 cultures, respectively, than in the control cultures. The corresponding PON values were 10.4-, 9.3-, and 7.2-fold higher than control PON values. The C/N ratio of the particulate (bacterial) production was 3.78 to 4.73 (Table 2). Maximum production of POC and PON demonstrated a linear correlation with the C/N ratio of the medium (Fig. 2A and B). Initial concentrations of DOC were 2.51 to 4.29 mg of per liter (Fig. 1E). During the incubation, consumption of DOC ranged from 0.24 (control) to 1.10 (C/N = 5) mg of C per liter per 25 h (Table 2). Between 25 and 33 h, DOC levels in three of the cultures increased slightly. The high DOC levels in the control cultures probably were due to contamination. However, the reduction of DOC levels in these cultures does not indicate increased DOC consumption relative to that of the +P cultures, in which natural DOC was the only carbon source. This suggests that bacteria in the control cultures did not utilize the additional DOC pool but only consumed naturally occurring DOC. The bacterial growth efficiency was higher in the C/N-manipulated cultures (45 to 53%) than in the unamended cultures (22 and 23%) (Table 2).

DCAA. During the incubation, DCAA levels in all cultures were reduced but after 17 or 25 h there was an accumulation of DCAA in four of the cultures (Fig. 1F). The average initial DCAA concentration was 2.92 μM. Maximum reduction of

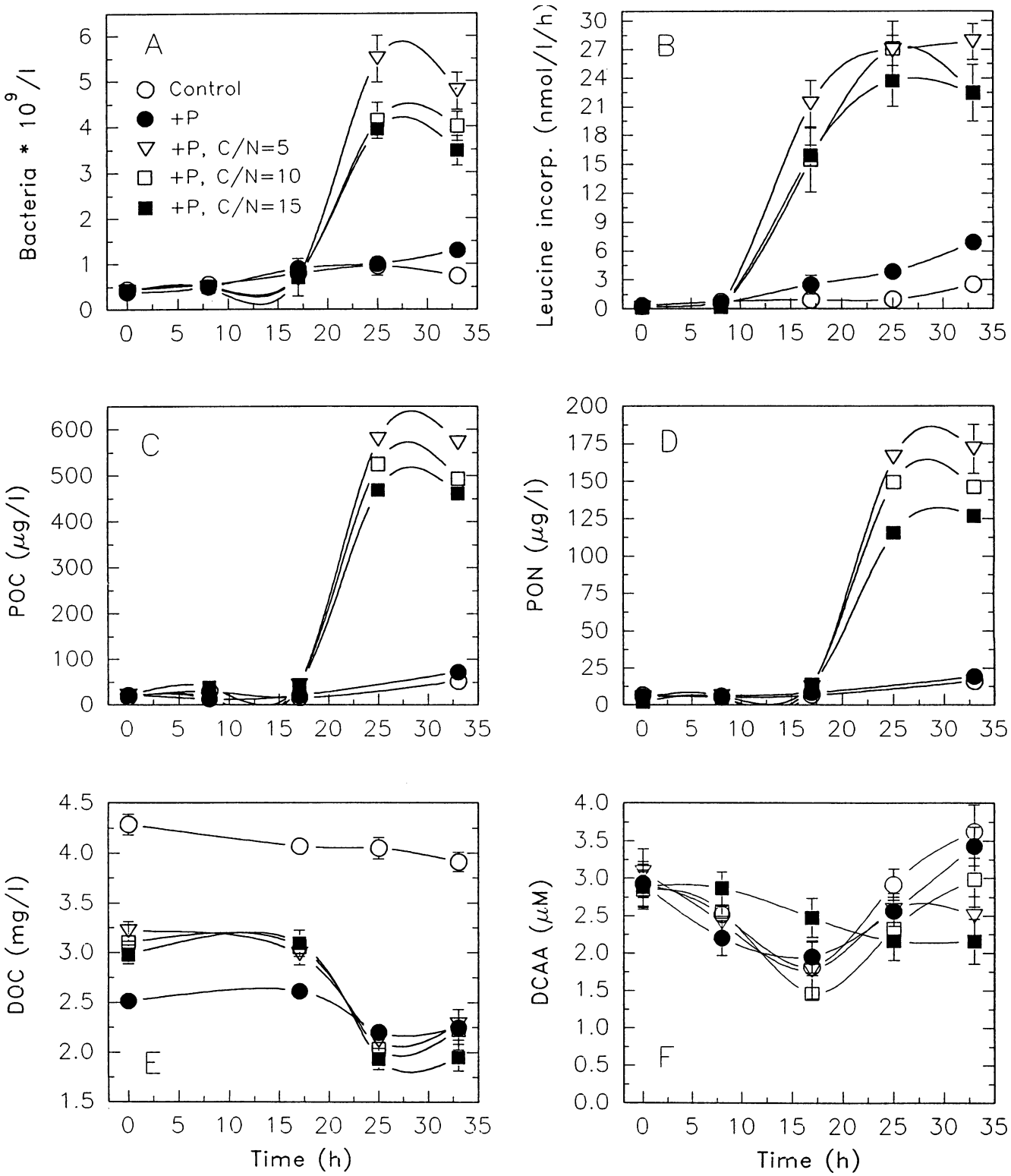

FIG. 1. Growth parameters of the Santa Rosa Sound batch cultures. (A) Bacterial density. (B) Incorporation of leucine. (C) Production of POC. (D) Production of PON. (E) Concentration of DOC. (F) Concentration of DCAA. Means ± standard deviations (SD) (n = 6) are shown.

DCAA in the cultures varied from 0.73 (C/N = 15) to 1.39 (C/N = 10) µM. During the initial 25 h, total uptake of DCAA was 1.8-, 2.6-, 3.7-, and 3.4-fold larger in the +P, C/N = 5, C/N = 10, and C/N = 15 cultures, respectively, than in the control cultures (Fig. 3). Dominant individual DCAA in the cultures were glycine, alanine, glutamic acid, and serine, representing an average of 38, 12, 11, and 5.9% of the total pools, respectively.

TABLE 2. Consumption of DOC, production of POC and PON, and bacterial carbon production[a]

Culture group	Rate of DOC consumption (μg of C/liter/25 h)[b]	Rate of POC production (μg of C/liter/25 h)	Growth efficiency (%)[c]	Rate of PON production (μg of N/liter/25 h)	C/N ratio[d]	Rate of bacterial carbon production (μg of C/liter/25 h)[e]
Control	237 ± 15	52.1 ± 4.3	22.0 ± 2.3	16.1 ± 0.1	3.78 ± 0.49	26.0 ± 5.1
+P	308 ± 54	71.9 ± 19.0	23.3 ± 7.4	19.1 ± 3.4	4.39 ± 0.26	57.8 ± 15.3
C/N = 5[f]	1,100 ± 85	578.2 ± 16.7	52.6 ± 4.3	166.0 ± 2.8	4.06 ± 0.34	394.3 ± 36.7
C/N = 10[f]	1,069 ± 116	524.8 ± 27.8	49.1 ± 5.9	149.4 ± 3.9	4.10 ± 0.79	328.0 ± 53.8
C/N = 15[f]	1,047 ± 70	468.9 ± 28.8	44.8 ± 4.1	115.6 ± 2.1	4.73 ± 0.25	315.1 ± 27.2

[a] Data are means ±1 SD (n = 6; triplicate samples from two carboys).
[b] Average initial DOC concentrations were 2.51 to 4.29 mg/liter.
[c] Produced POC as a percentage of consumed DOC.
[d] POC relative to PON (molar ratios).
[e] Based on incorporation of leucine, assuming that leucine makes up 8.5% of bacterial proteins, that 63% of the bacterial dry weight was protein, and that 54% of the dry weight was carbon (24).
[f] Contained phosphate.

DFAA. In the control and +P cultures, concentrations of DFAA varied from 10 to 249 nM, with the lowest concentrations occurring at 8 and 33 h (Fig. 4A). In the C/N-manipulated cultures, to which 3.33 μM alanine was added, a significant decline in the DFAA levels occurred after 17 h. At 25 and 33 h, DFAA concentrations in these cultures were below 100 nM. Assimilation of DFAA peaked at 17 h (+P and C/N-manipulated cultures) or at 25 h (control) (Fig. 4B). Relative to the control cultures, 0.85-, 11.5-, 11.6-, and 9.0-fold-higher assimilations occurred in the +P, C/N = 5, C/N = 10, and C/N = 15 cultures, respectively, during the initial 25 h (Fig. 3). Respiration of the DFAA was variable (data not shown). In the C/N-manipulated cultures, respiration was 76 to 89% during the initial 8 h. Later it declined to 65 to 75% (17 h) and 27 to 36% (25 and 33 h). In the control cultures, respiration was initially 28 to 34%, but it fluctuated between 48 and 70% after 17 h. In the +P cultures, the respiration varied between 24 and 35% throughout the incubation period. The most abundant DFAA were glycine, lysine, serine and ornithine, constituting an average of 29, 27, 19, and 13%, respectively, of the total pools.

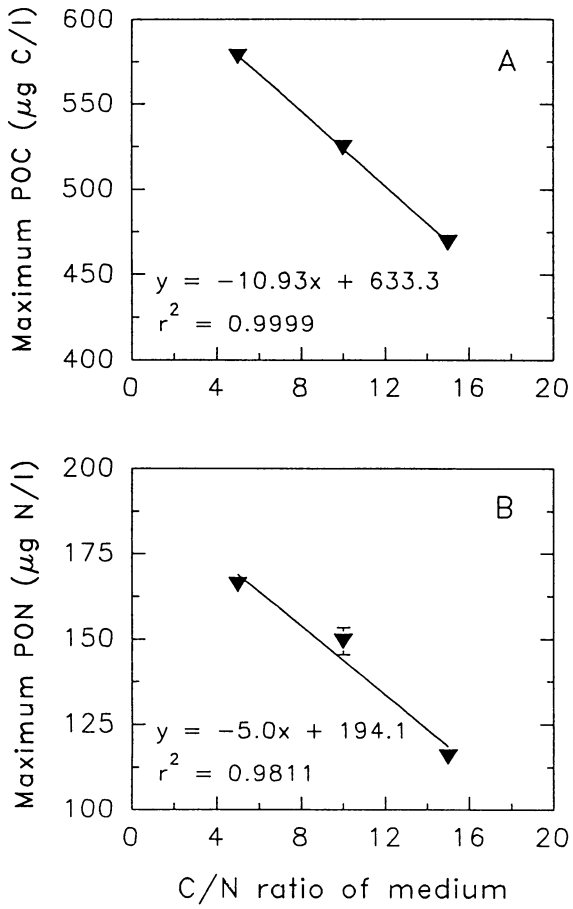

FIG. 2. Maximum POC (A) and PON (B) production after 25 h of incubation versus C/N ratio of the medium. Means ± SD (n = 6) are shown.

D-DNA. In the control and +P cultures, the concentrations of D-DNA decreased from initial levels of 12 and 6 μg/liter, respectively, to 2 to 3 μg/liter at 17 and 33 h (Fig. 4C). In the C/N-manipulated cultures, D-DNA concentrations varied between 1.5 and 3.8 μg/liter. Bacterial assimilation of D-DNA increased in all cultures, ranging from an initial level of 75 pg/liter/h (160 pg/liter/h in the +P cultures) to 400 to 550 pg/liter/h (300 pg/liter/h in the C/N = 5 cultures) at 33 h (Fig. 4D). Integrated assimilation rates (0 to 25 h) demonstrate that the DNA uptake increased with the addition of phosphate and with an increasing C/N ratio (Fig. 3).

Inorganic nitrogen. In the control and +P cultures, NH_4^+ was reduced from an initial concentration of about 3.3 μM to less than 2.0 μM at 25 h (Fig. 4E). In the C/N-manipulated cultures, to which 3.3 to 16.6 μM NH_4^+ was added, a significant uptake reduced the NH_4^+ concentration to about 1.3 μM at 25 h, except in the C/N = 5 cultures, in which a minimum of 6.3 μM NH_4^+ occurred. After 25 h, NH_4^+ was released in all cultures.

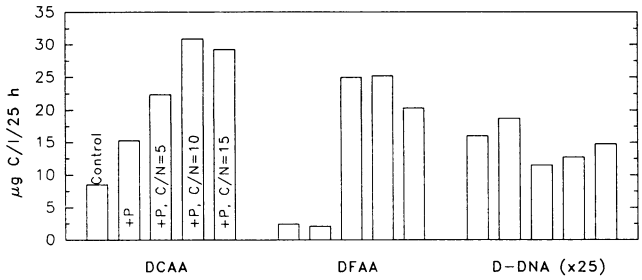

FIG. 3. Integrated carbon incorporation (0 to 25 h) from DCAA, DFAA, and D-DNA. Means ± SD (n = 6) are shown. +P, phosphate added.

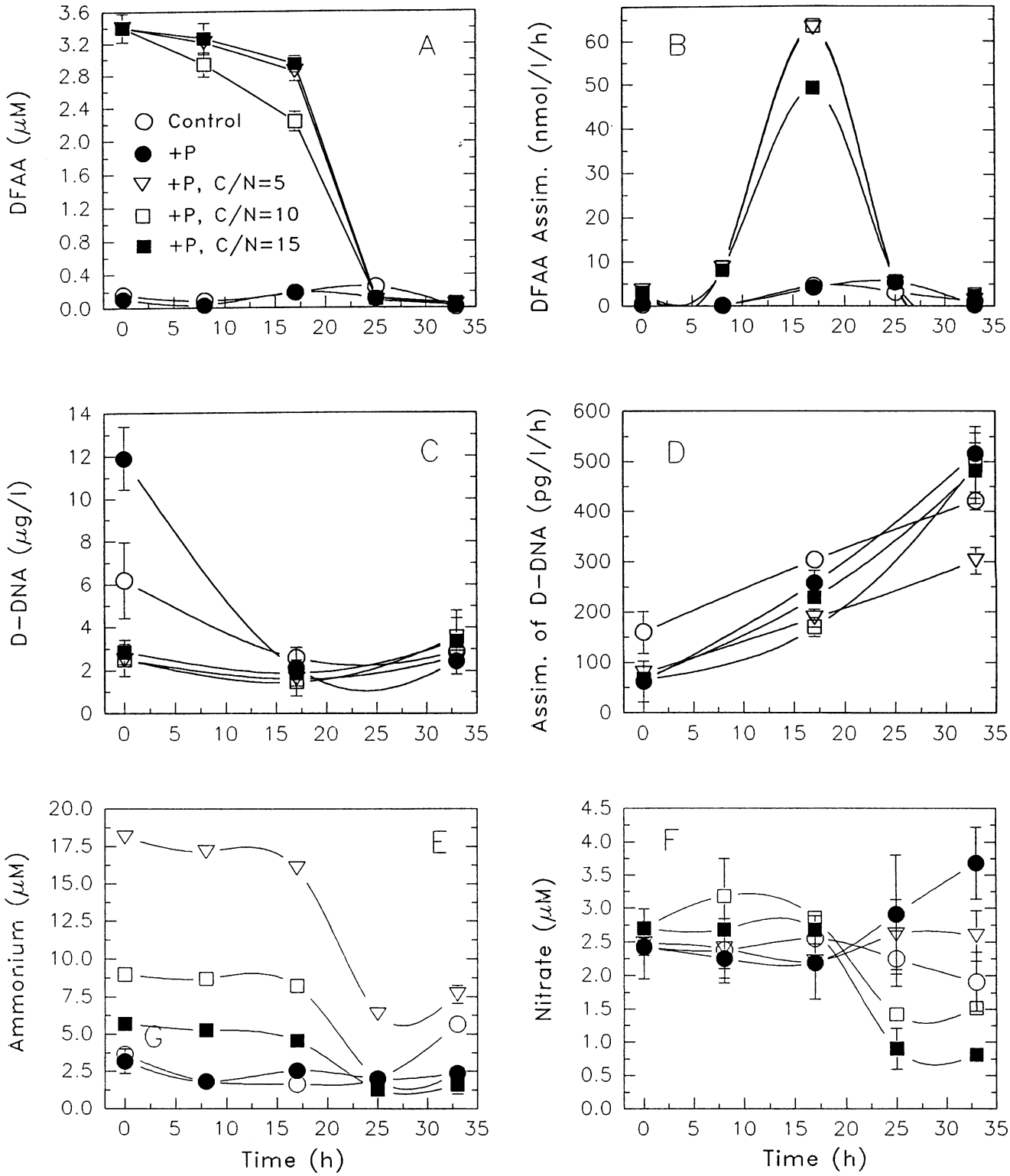

FIG. 4. Utilization of nutrients in the Santa Rosa Sound batch cultures. (A) Concentration of DFAA. (B) Assimilation of DFAA. (C) Concentration of D-DNA. (D) Assimilation of D-DNA. (E) Concentration of ammonium. (F) Concentration of nitrate. Means = SD ($n \pm 6$) are shown.

TABLE 3. Net incorporation of C and N from assimilation of DCAA, DFAA, DNA, NO_3^-, and NH_4^+ relative to bacterial C and N production, measured as POC and PON[a]

Culture group	Net incorporation of carbon (μg of C/liter/25 h)[b]	Net C incorporation as % of POC production	Net incorporation of nitrogen (μg of N/liter/25 h)		Net organic N incorporation as % of PON production	Net inorganic N incorporation as % of PON production	Total net N incorporation as % of PON production
			Organic N[b]	Inorganic N[c]			
Control	12.9 ± 2.3	24.7 ± 4.9	6.1 ± 1.9	9.6 ± 4.2	37.9 ± 11.9	60.0 ± 26.4	97.9 ± 52.9
+P	19.6 ± 15.6	27.3 ± 22.9	9.3 ± 7.6	25.5 ± 4.9	48.7 ± 40.5	133.6 ± 34.8	182.3 ± 155.6
C/N = 5[d]	48.7 ± 12.5	8.4 ± 2.2	22.9 ± 7.1	163.1 ± 49.2	13.8 ± 4.3	98.3 ± 29.7	112.1 ± 48.6
C/N = 10[d]	57.6 ± 10.3	11.0 ± 2.1	27.1 ± 8.2	116.7 ± 17.8	18.2 ± 5.5	78.1 ± 12.1	96.3 ± 32.6
C/N = 15[d]	51.3 ± 5.7	10.9 ± 1.4	24.2 ± 4.3	86.7 ± 31.5	20.9 ± 3.8	75.0 ± 27.2	95.9 ± 38.8

[a] Data are means ±1 SD (n = 6; triplicate samples from two carboys).
[b] Sum of individual contributions of DCAA, DFAA, and D-DNA.
[c] Sum of NO_3^- and NH_4^+.
[d] Contained phosphate.

The initial concentration of NO_3^- in all cultures was about 2.5 μM (Fig. 4F). In the control and C/N = 5 cultures, no major changes occurred during the incubation. In the C/N = 10 and C/N = 15 cultures, in which the lowest NH_4^+ concentrations occurred, an uptake of NO_3^- was observed. In the +P cultures, NO_3^- was released.

Carbon budget. Bacterial carbon production, determined from the incorporation of leucine, was on average 66% of the POC production (Table 2). Apparently the applied factors for conversion of leucine incorporation into bacterial carbon production (24) underestimated the actual POC production. Net incorporation of carbon from DCAA, DFAA, and D-DNA ranged from 13 (control) to 58 (C/N = 10) μg of C per liter per 25 h (Table 3). This incorporation corresponded to 8.4 to 11% (C/N-manipulated cultures) and 25 and 27% (control and +P cultures) of the bacterial carbon production (POC). In the control and +P cultures, the average levels of relative significance of carbon from DCAA, DFAA, and D-DNA were 72, 15, and 13%, respectively. In the C/N-manipulated cultures, in which incorporation of DFAA was greater because of the addition of alanine, the values were 52, 45, and 3%. Carbon budgets for the incubation period from 0 to 25 h are illustrated in Fig. 3.

Nitrogen budget. Organic nitrogen (from DCAA, DFAA, and D-DNA) sustained on average 38 and 49% of the bacterial N demand in the control and +P cultures (Table 3). In the C/N-manipulated cultures, the level of incorporation of organic nitrogen was about 4-fold higher than that in the control cultures. Because of the considerably higher level of bacterial production, however, the relative importance of DCAA, DFAA, and D-DNA was less. In C- and N-amended cultures, organic nitrogen supplied from 14 (C/N = 5) to 21% (C/N = 15) of the bacterial nitrogen demand. The relative individual significance of DCAA, DFAA, and D-DNA as nitrogen sources did not differ from the relative significance of the same substances in their carbon contribution (see above).

Inorganic nitrogen (NH_4^+ and NO_3^-) was found to be a more important bacterial nitrogen source than organic nitrogen (DCAA, DFAA, and D-DNA) in all cultures (Table 3 and Fig. 5). In the control and +P cultures, inorganic N made up 60

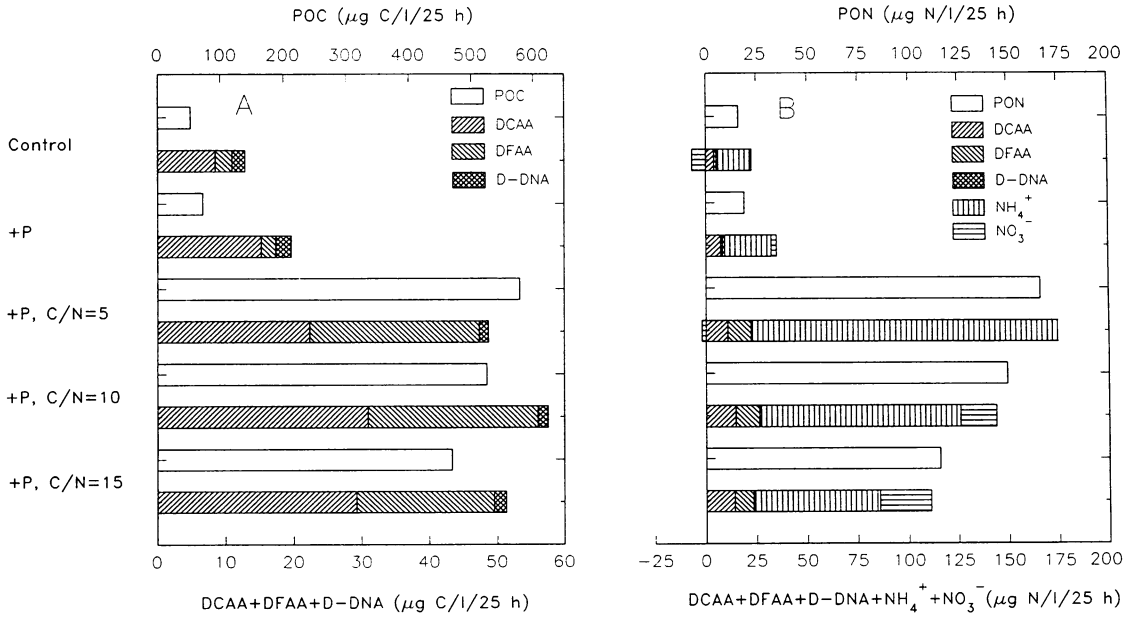

FIG. 5. Carbon (A) and nitrogen (B) budgets of the Santa Rosa Sound batch cultures during the initial 25-h growth period. Rates of assimilation of DCAA, DFAA, and D-DNA are integrated values for 0 to 25 h. Production of POC and PON and uptake or release of NH_4^+ and NO_3^- are calculated from the differences in concentrations at 0 h and 25 h. +P, phosphate added.

and 134% of the total bacterial N incorporation (PON), respectively. The inorganic N made up 61 and 73% of the total net nitrogen incorporation from DCAA, DFAA, D-DNA, NH_4^+, and NO_3^-. In the C/N-manipulated cultures, incorporation of inorganic N was 75 (C/N = 15) to 98% (C/N = 5) of the PON production. This corresponded to 81 (C/N = 10), 78 (C/N = 15), and 88% (C/N = 5) of the total incorporation of DCAA, DFAA, D-DNA, NH_4^+, and NO_3^-.

The measured net nitrogen incorporation sustained most (96 to 98%) of the bacterial nitrogen demand in three of the cultures, but in two sets of cultures (+P and C/N = 5) the nitrogen incorporation exceeded the bacterial nitrogen production (Table 3). However, considering the large experimental variation of the nitrogen incorporation in these two cultures, the incorporation was not statistically different from the observed PON production.

DISCUSSION

Manipulations of nutrient regimens of the bacterial cultures demonstrated that the bacterial utilization of DCAA, DFAA, and D-DNA was influenced by the availability of other carbon and nitrogen sources and by phosphorus. The mechanisms regulating the assimilation of DCAA, DFAA, and D-DNA appeared, however, to be variable in the different cultures.

Incorporation of DCAA, D-DNA, and DFAA versus bacterial production. DCAA were assimilated by the bacteria in all cultures. The higher rates of DCAA incorporation in the C/N-manipulated cultures, compared with those in the control and +P cultures, may be related to the 10-fold-higher level of bacterial production, which introduced a larger metabolic activity, and to an increased nitrogen deficit with the increasing C/N ratio. The higher rate of DCAA incorporation at C/N ratios of 10 and 15, relative to that at a C/N ratio of 5, may also reflect the changing ratio between inorganic N and amino-N with the increasing C/N ratio. With an increase in the C/N ratio from 5 to 15, the alanine/NH_4^+ ratio increased from 0.20 to 1 (Table 1). A portion of the alanine may have been used for synthesis of proteins with little de novo amino acid synthesis, if other amino acids were available. These amino acids could originate from DCAA.

Incorporation of DCAA sustained 4 to 6% and 6 to 12% of the POC and PON production, respectively, at C/N ratios between 5 and 15. In control and +P cultures, DCAA met 16 to 21% and 25 to 38% of the needs for POC and PON production, respectively. The contribution of DCAA carbon in the unamended cultures was comparable to that observed in previous studies of Santa Rosa Sound, in which DCAA were found to sustain up to 10% (11) and 21% (18) of the POC production. As for the bacterial nitrogen incorporation, the DCAA contributions in the present study (27 to 35% of the bacterial N incorporation in the control and +P cultures) are intermediary relative to values of up to 15% (11) and 112% (18) of the PON production previously measured in Santa Rosa Sound. The importance of DCAA nitrogen in the present study was moderately greater than that observed by Keil and Kirchman (14), who obtained values of 1 to 20% after adding about 100 nM algal protein to estuarine water samples. In eutrophic lake water, DCAA may be more important as bacterial carbon and nitrogen sources than they are in marine waters, and occasionally they may be the dominant carbon source (22). In our calculations it was assumed that reduction of the DCAA represented incorporation into the bacteria. Recent data indicate that about one-fifth of the carbon of proteins assimilated by estuarine bacteria can be respired (14). Respiration of DCAA was not included in our study. If a portion of the assimilated DCAA was respired, then the calculated DCAA incorporation rates are overestimated. In our study, at most half of the ambient DCAA were consumed by the bacteria. In other studies a similar partial utilization of DCAA has been observed (14). Apparently a portion of natural DCAA is less degradable by bacteria or maybe even refractory, e.g., if it is sorbed to colloids and clays (14).

D-DNA appeared to serve mainly as a nitrogen source for the bacteria, as incorporation of D-DNA increased with the C/N ratios of the C/N-manipulated cultures. At a C/N ratio of 5, the incorporation of D-DNA was 70% of the rate measured in the control and +P cultures. At C/N ratios of 10 and 15, the D-DNA incorporation had increased to 80 and 92%, respectively, of the control values. Although D-DNA nitrogen in all cultures provided less than 0.7% of the PON production, D-DNA nitrogen may have been valuable to the bacteria, e.g., if D-DNA products (nucleotides) were salvaged into bacterial nucleic acids (20). D-DNA was found to sustain up to 5 to 12% of the bacterial carbon and nitrogen production, respectively, in other batch cultures of marine bacteria (11, 18).

DFAA incorporation was unaffected by the addition of phosphate. Therefore, the higher level of bacterial production in the +P cultures, relative to that of the control cultures, was not caused by increased DFAA incorporation. The reason for this may be sought in the small DFAA pools (<200 nM in most cultures) that may have limited the assimilation rate. In the C/N-manipulated cultures, addition of 3.3 μM alanine led to an approximately 10-fold-larger amount of DFAA incorporation. The increased alanine concentration did not change the relative contributions of amino carbon and nitrogen to the bacteria. Thus, in all cultures uptake of DFAA and added alanine sustained about 4 and 8% of the bacterial POC and PON production, respectively. In the C/N = 15 cultures, in which the bacterial production was limited by nitrogen (see below), DFAA probably were a nitrogen source for the bacteria, but it cannot be estimated whether the bacteria in the other cultures mainly used carbon or nitrogen from the assimilated amino acids. Generally DFAA appear to be major nutrients of aquatic bacteria, but their importance seems to be variable. DFAA carbon and nitrogen have been reported to sustain 5 to 100% and 8 to 246% of the bacterial carbon and nitrogen demands, respectively, in marine waters (11, 13, 23). This considerable variation in the importance of DFAA to bacteria may be related to the composition and quality of available DOC and DON (22). The unrealistically large amount of nitrogen incorporated relative to the bacterial nitrogen demand occasionally measured, as indicated above, will be discussed later.

Substrate composition and bacterial POC and PON production. In the +P cultures, a 1.2- to 1.4-fold-higher level of bacterial PON and POC production and a 2.2-fold-higher level of incorporation of leucine, relative to the control levels, were observed (Table 2). This suggests that the bacteria in Santa Rosa Sound at the sampling time were limited by phosphorus. The stimulated production was reflected in a doubling of the DCAA incorporation, while incorporation of both DFAA and D-DNA remained unchanged. If D-DNA was a source of phosphorus for the bacteria, as has been observed in the case of phosphorus-limited microorganisms (27), a reduced uptake of D-DNA in the +P cultures might have been expected. The unchanged D-DNA uptake suggests rather that D-DNA was a source of C or N or both. The two-fold-higher level of DCAA assimilation in the +P cultures met the need for one-third of the additional POC and PON production, indicating that carbon and nitrogen compounds other than those studied here were dominant nutrients for the bacteria.

In the C/N-amended cultures glucose and ammonium were the major carbon and nitrogen sources for the bacteria. To determine if the added nutrients provided sufficient carbon and nitrogen to sustain the observed bacterial POC and PON production, we created a simple model relating the substrate C/N ratio (organic as well as inorganic nutrients included) to the bacterial C/N ratio and carbon and nitrogen growth efficiencies. The bacterial carbon and nitrogen growth efficiencies (Y_C and Y_N) can be determined from the consumption of DOC (ΔDOC) and dissolved nitrogen (ΔDN, ΔDON + ΔDIN) and the production of POC (ΔPOC) and PON (ΔPON) by the following equations:

$$Y_C = \frac{\Delta POC}{\Delta DOC} \text{ and } Y_N = \frac{\Delta PON}{\Delta DN} \quad (1 \text{ and } 2)$$

The bacterial C/N ratio, $(C/N)_B$, can be calculated as follows:

$$\left(\frac{C}{N}\right)_B = \left(\frac{\Delta POC}{\Delta PON}\right) \text{ or } \Delta POC = \left(\frac{C}{N}\right)_B \cdot \Delta PON \quad (3 \text{ and } 4)$$

On the basis of equations 1 to 4 the consumption of DOC can be expressed as

$$\Delta DOC = \frac{\left(\frac{C}{N}\right)_B \cdot Y_N \cdot \Delta DN}{Y_C} \quad (5)$$

The C/N ratio of the substrate utilized by the bacteria, $(C/N)_S$, can be determined as follows:

$$\left(\frac{C}{N}\right)_S = \frac{\Delta DOC}{\Delta DN} \quad (6)$$

By inserting equation 5 into equation 6, the following relationship between $(C/N)_B$ and $(C/N)_S$ is obtained:

$$\left(\frac{C}{N}\right)_S = \left(\frac{C}{N}\right)_B \cdot \frac{Y_N}{Y_C} \quad (7)$$

From equation 7 the C/N ratio of the substrate taken up by the bacteria can be determined, if the bacterial C/N ratio and the bacterial carbon and nitrogen growth efficiencies are known. We did not measure Y_N. However, if Y_N is assumed to be 100%, i.e., $(C/N)_S = (C/N)_B/Y_C$, the maximum value of $(C/N)_S$ allowing production of bacterial biomass of a given $(C/N)_B$ can be calculated. Using the data in Table 2, one finds that the $(C/N)_S$ of the C/N = 5 cultures must have been 7.7 or less (if Y_N was <100%) to produce bacteria with the measured C/N ratio. In the C/N = 10 and C/N = 15 cultures, the $(C/N)_S$s must have been $\leq$8.4 and $\leq$10.6, respectively. In the control and +P cultures, the values were $\leq$17.2 and $\leq$18.8. These estimated $(C/N)_S$s indicate that in the C/N = 5 cultures, the manipulated $(C/N)_S$ was sufficiently low to sustain the measured production. In support of this, only 66% of the added NH_4^+ was taken up in these cultures (Fig. 4E). In the C/N = 10 and C/N = 15 cultures, on the other hand, the added nutrients introduced a nitrogen deficit. The nitrogen deficit probably was met by naturally occurring nitrogen compounds such as DCAA and NO_3^-. At a C/N ratio of 15 the highest level of assimilation of NO_3^- occurred, and this assimilation sustained 23% of the PON production. The $(C/N)_S$s of 7 to 8 of natural dissolved organic matter in Santa Rosa Sound (16, 17) theoretically was sufficiently low to support the $(C/N)_S$s of $\leq$17.2 and $\leq$18.8 in the control and +P cultures. However, since only a portion of the natural DOC and DON can be used by bacteria, the actual $(C/N)_S$ may have differed from 7 and 8.

The correlation between maximum bacterial carbon and nitrogen content and increased $(C/N)_S$ (Fig. 2) indicates that the bacterial production in the C/N-amended cultures was regulated by the availability of nitrogen. The correlations also suggest that with the tested C/N ratios, a linear relationship between bacterial biomass and ambient C/N ratios can be predicted. A similar observation was made by Goldman and Dennett (7) when they grew bacteria on glucose, NH_4^+, and amino acids and by Kroer (16), who added NH_4^+ to natural seawater.

Production of inorganic nitrogen, typically NH_4^+, is expected to occur when bacteria assimilate dissolved organic matter with a C/N ratio below the $(C/N)_B$, but the bacterial carbon growth efficiency must also be considered. Different studies indicate that at a $(C/N)_S$ of <10, production of NH_4^+ will occur (6, 25). Kirchman et al. (15) suggested that bacterial mineralization of nitrogen (as NH_4^+) is expected to occur, if DFAA supply more than 80% of the bacterial nitrogen demand. In our experiments, there was a release of NH_4^+ in the control and C/N = 5 cultures (between 25 and 33 h) and a release of NO_3^- in the +P cultures (between 17 and 33 h). The incorporation of leucine indicated that the bacteria were still growing at 33 h in these cultures. Thus, the release of inorganic nitrogen was not due to cell mortality. The production of NH_4^+ at a C/N ratio of 5 was expected, as the bacteria required a $(C/N)_S$ of 7.7 or less. In the control and +P cultures, a nitrogen mineralization could not be predicted, as the C/N ratio of organic compounds taken up by the bacteria was unknown. However, the production of both NH_4^+ and NO_3^- suggests that the $(C/N)_S$ was substantially below the estimated values of 17.2 and 18.8. The NO_3^- production indicates that a nitrification occurred. The largest increase of NO_3^-, about 1.5 μM (+P cultures), was greater than nitrification rates generally observed in open, marine waters, but it was within the range of rates measured in lakes (8, 28).

The bacterial carbon conversion efficiency increased from 22 to 23% in the unamended cultures to 45 to 53% in the C/N-amended cultures. The growth efficiencies of the unamended cultures agreed with values of 26 to 33% previously reported for Santa Rosa Sound (2, 16), and they fall within the range of growth efficiencies typically reported for aquatic ecosystems (3, 19). In natural waters, 70 to 80% of the glucose and 40 to 60% of the DFAA taken up by bacteria are typically incorporated (5, 9). Since the substrate of the C/N-manipulated cultures was dominated by glucose and DFAA (mainly alanine), the higher carbon conversion efficiency in these cultures was expected. In the unamended cultures, the bacterial production was due to organic compounds other than DCAA, DFAA, and D-DNA, as these substances made up only about 26% of the POC production. The release of inorganic nitrogen in these cultures suggests that the low carbon conversion efficiency was not caused by an unfavorable $(C/N)_S$. More likely, the inorganic nitrogen production indicates that the bacterial growth was limited by assimilatable carbon compounds.

The high initial level of DFAA respiration in the C/N-manipulated cultures (76 to 89%) indicates that during the early cell production period amino acids primarily were used for energy production or possibly served as a source of nitrogen rather than carbon. Later in the experiments, the respiration declined to the level of 27 to 36% measured in the +P cultures and in some of the control cultures.

Carbon and nitrogen balance. Carbon budgets for the initial 25 h demonstrate that the DOC consumption (1,047 to 1,100 μg of C per liter) in the C/N-manipulated cultures was largely identical to the addition of glucose and alanine carbon (1,080 μg/liter) (Table 2). Relative to this, the level of incorporation of DCAA, DFAA, and D-DNA carbon in these cultures was low (49 to 58 μg/liter), and this incorporation made up less than 11% of the POC production (Fig. 5 and Table 3). The level of incorporation of DCAA, DFAA, and D-DNA in the control and +P cultures was lower than that in the C/N-manipulated cultures. However, because of the lower levels of bacterial production in these two types of cultures, DCAA, DFAA, and D-DNA met the need for about 26% of the POC production. This contribution is somewhat below the 40 to 90% sustenance of bacterial carbon production by DCAA, DFAA, and D-DNA measured in other batch cultures of marine bacteria (11, 18). This discrepancy is mainly caused by a low level of DFAA incorporation by the Santa Rosa Sound bacteria.

The nitrogen budgets show that NH_4^+ and NO_3^- were major bacterial nitrogen sources, providing 60 to 134% (control and +P cultures) and 75 to 98% (C/N-amended cultures) of the PON production (Fig. 5 and Table 3). The equivalent values of DON (DCAA, DFAA, and D-DNA) were 38 to 49% and 14 to 21%, respectively. Results for the unamended cultures indicate that actively growing bacteria in Santa Rosa Sound may be of greater importance as consumers of inorganic nitrogen than as producers of it. The significance of bacteria as sinks of inorganic nitrogen has been shown in several studies, e.g., those of Tupas and Koike (26) and Keil and Kirchman (13), who found that NH_4^+ often may sustain at least half of the bacterial nitrogen demand. Other major nitrogen sources in their studies were DFAA and DCAA.

The apparently high rate of nitrogen incorporation observed in the +P cultures (up to 338% of the bacterial nitrogen demand) in our study and occasionally in other studies (11, 13, 18, 22) is probably caused by the release of various nitrogen compounds not accounted for in the studies. Among possible nitrogen products released by marine bacteria, methylamines (11, 29) and urea (10, 21) have been found to be important. The importance of urea as a possible bacterial excretion product is discussed by Kroer et al. (18).

In conclusion, the experiments demonstrated that bacterial utilization of the studied organic nitrogen compounds was regulated by the availability of other carbon and nitrogen sources and by the substrate C/N ratio. Thus, with the addition of supplementary carbon and nitrogen sources (glucose, alanine, and NH_4^+) at biologically realistic C/N ratios, the incorporation of naturally occurring DCAA increased significantly, while the incorporation of D-DNA decreased with increasing substrate C/N ratios. Changing the substrate C/N ratio by addition of the carbon and nitrogen compounds also influenced bacterial growth, as a 2- to 2.5-fold-higher growth yield, relative to that of the unenriched cultures, was obtained. The present results, and the results obtained by Kroer et al. (18), show that DCAA at natural substrate concentrations can be a more important C and N source for marine bacteria than previously observed, but their significance appears to depend on the availability of other organic and inorganic compounds.

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